being fused to ubiquitin at the N-terminus of ubiquitin, wherein the ubiquitin fusion protein is immunogenic for the non-ubiquitin self-epitopes contained therein;

b) administering the fusion protein of step a) to an animal under conditions appropriate for the stimulation of an immune response, thereby stimulating an immune response to the non-ubiquitin self-epitopes.

In the Specification

Please delete the section entitled "Government Support" located after the title in the application.

REMARKS

Provisional Obviousness-type Double Patenting Rejection

Claims 82-84, 88-91, and 96-100 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Claims 76-79, 84-85, 87-90 and 93 of co-pending Application No. 09/026,276. Claims 76-79, 84-85, 87-90 and 93 of co-pending Application No. 09/026,276, were canceled in an Amendment filed with the Patent Office on January 24, 2001, thus obviating this provisional rejection.

Rejection Under 35 USC 112, First Paragraph

Claims 82-89 and 95-100 have been rejected under 35 USC 112, first paragraph. More specifically, the Patent Office states:

> The specification ... does not reasonably provide enablement for methods for stimulating all immune responses utilizing ubiquitin fused to all self antigens. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. The aforementioned claims broadly encompass an infinite

> array of ubiquitin fusion proteins which contain an equally limitless number of self epitopes. ... the quantity of experimentation to determine which self-epitopes to be fused to the ubiquitin to achieve the desired immune response which are encompassed by the scope of the claims is practically infinite and the guidance provided by the specification is minimal. Coupled with the high degree of unpredictability of the art it would require undue experimentation to determine how to use all the possible ubiquitin/self antigen fusion proteins encompassed by the scope of the claims.

This rejection is respectfully traversed. Applicants provide two independent examples of the generation of an immune response to two different self-proteins (GnRH and growth hormone) by incorporating self-epitopes derived from those proteins in a ubiquitin-fusion protein and using the ubiquitin fusion protein to induce an immune response to the self-epitope. Further, Applicants provide several working examples of ubiquitin fusion proteins (pX548, pX549, and pX552, see Tables 3 and 4 for experimental results) which have the self-epitopes fused at different positions within the ubiquitin molecule (C-terminal to ubiquitin, N-terminal to ubiquitin, and internally) to produce ubiquitin fusion proteins which are immunogenic for the self-epitopes incorporated therein. The determination of additional sites of epitope fusion which preserve the native secondary and tertiary structure of ubiquitin, as necessary for the present invention is within the ability of one of skill in the art given the guidance provided within the specification and the additional factors 1) The three-dimensional structure of ubiquitin has been determined by X-ray crystallography and its small size makes it amenable to molecular modeling, and 2) ubiquitin fusions are readily expressed and purified in prokaryotic systems such as *E. coli*, in soluble form. Further, a significant amount of guidance is given in the Specification as to additional methods of enhancing the immunogenicity of the self-epitope(s) in the context of the ubiquitin fusion protein. These include additional epitopes (B-cell and T-cell) for incorporation in the fusion protein, and additional modifications of the ubiquitin

fusion proteins. The field of immunogen synthesis is highly developed and other methods of experimentally inducing an immune response to a self-antigen are well known in the art (for example see Paul Fundamental Immunology, 3rd Edition., P. 1038, last paragraph, to 1039 first paragraph, and Mouritsen et al., both cited by the Patent Office). Thus, selection of a self-epitope for incorporation into the ubiquitin fusion protein can be made by one of skill in the art through no more than routine experimentation.

Regarding the unpredictability in the art, the Patent Office states:

> Said claims are all drawn to inducing an "immune response" to a self antigen in an animal and hence, would be considered "autoimmune response" by definition. Autoimmune responses are quite complex and not fully understood by those skilled in the art. Additionally, many autoimmune responses are deleterious to the health and well being of an animal ... Consequently, it would be impossible to predict what effect an immune response to a given self antigen would have on an animal.

Although naturally occurring autoimmune responses are complex and not fully understood, and thus unpredictable, methods of experimentally inducing an immune response to a self-antigen are well known in the art, for example see Paul Fundamental Immunology, 3rd Edition., P. 1038, last paragraph, to 1039 first paragraph, and Mouritsen et al., both cited by the Patent Office. Regarding statements made by the Patent Office regarding the present invention causing possible deleterious effects in an animal, Applicants are not required to demonstrate that the invention is completely safe (MPEP 2164.01(c) second paragraph). Possible, deleterious side-effects to an animal which arise from an immune response to a self-antigen, do not effect the patentability of the claims in that they do not necessarily preclude use of the invention. Often such side effects are preferable to side effects which arise from alternative treatments, or a lack of treatment entirely. For example,

physical side effects arise from many such treatments (e.g., physical castration of pigs results in poorer growth and higher fat percentage, many cancer therapies produce deleterious side effects to the patient) and are not prohibitive to use in that they are preferable to a lack of treatment altogether.

Rejection Under 35 USC 112, Second Paragraph

Claims 87 and 91-92 have been rejected under 35 USC 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection has been obviated by amendment of the claims. Regarding Claim 87, the term "ubiquitin moiety" is objected to as having insufficient antecedent basis. In response, the term "moiety" has been deleted. Regarding Claims 91 and 92, the Patent Office states that it is unclear what is being administered. In response, Claim 91 has been amended to specifically recite "administration of the ubiquitin fusion protein". Further regarding Claims 91 and 92, the Patent Office states that it is unclear what constitutes "similarity". This rejection has been obviated by amendment of Claim 91 to recite "the physiological consequence of administration of the ubiquitin fusion protein to the animal is immunocastration of the animal."

Rejection Under 35 USC 102(b)

Claims 82, 84, 88, 89, and 97-100 have been rejected under 35 USC 102(b) as being anticipated by Vannier et al. More specifically the Patent Office states:

> Vannier et al. disclose the expression of the extracellular domain of human follicle stimulating hormone receptor (hFSHR) in *E. coli* as a ubiquitin fusion protein, (see abstract). Vannier et al. also disclose that immunization of mice with said fusion protein (Ub-hFSHR) resulted in high affinity anti-receptor monoclonal antibodies. Immunization of monkeys with said fusion protein also induced the formation of anti-receptor antibodies (see page 13359

> second paragraph, and page 1365, last paragraph). Consequently, the disclosure of Vannier et al. anticipates all the limitations of the instant claims.

The rejection of Claims 97, 99 and 100 is respectfully traversed. Although the disclosure of Vannier et al., and also the disclosure of Loosfelt et al. (referenced as a source of information for the construction of this fusion protein, copy enclosed) do not specifically disclose the construction of this fusion protein, one of skill in the art would reasonably conclude from the nomenclature used (Ub-hFSHR(23-358)) that the fusion protein contains amino acids 23-358 of hFSHR fused to the C-terminus of ubiquitin. Claim 97 recites the limitation that the ubiquitin fusion protein comprises ubiquitin fused to an epitope containing segment comprising <u>two or more identical non-ubiquitin self-epitopes</u>. The identical epitopes of the present invention are indicated by the presence of repeating identical sequences of 10 or more amino acids (for example, see page 44, line 17-20 of the instant application, which refers to multiple copies of the GnRH epitope). Vannier et al. teaches ubiquitin fused to amino acids 23-358 of FSHR, at the C-terminus. The presence of multiple epitopes in the FSHR sequences of the Vannier et al. fusion protein does not denote the presence of identical epitopes. As stated in the MPEP:

> "In relying on the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464

The Patent Office has not supplied any such basis in fact or technical reasoning. There are no repeated sequences in the FSHR which qualify as identical epitopes. Therefore, Vannier et al. does not teach ubiquitin fused to two or more identical epitopes and does not anticipate Claim 97.

Claim 99 specifically recites "the epitope-containing segments being fused to ubiquitin at fusion sites selected from

the group consisting of the N-terminus and an internal fusion site". Claim 100 specifically recites "the epitope-containing segment being fused to ubiquitin at the N-terminus of ubiquitin". As the ubiquitin fusion protein of Vannier et al. is fused to the C-terminus of ubiquitin, this does not meet all of the limitations of Claims 99 and 100, and therefore does not anticipate either claim.

In response to the rejection of Claim 98, said claim has been amended to more clearly recite the invention, and now recites the limitation:

> "providing a ubiquitin fusion protein comprising ubiquitin fused to two or more [non-contiguous] epitope-containing segments <u>at non-contiguous locations within ubiquitin</u>,

Vannier et al. teaches ubiquitin fused to amino acids 23-358 of FSHR, at the C-terminus. This does not meet the above quoted limitation of two or more epitope-containing segments being fused to ubiquitin at non-contiguous locations within ubiquitin, and therefore does not anticipate amended Claim 98.

Claims 82, 84, and 97-100 have been rejected under 35 USC 102(b) as being anticipated by Mouritsen et al. (WO 95/05849). More specifically, the Patent Office states:

> Mouritsen et al. disclose the attachment of one or more T cell epitopes into the highly conserved self protein ubiquitin. ... Injection of said fusion proteins into mice elicited a strong antibody response to the fusion protein. Consequently, the disclosure of Mouritsen et al. anticipates all the limitations of the instant claims.

This rejection has been obviated by amendment of the Claims to more specifically recite the invention. Amended Claims now recite that the immune response is directed toward a <u>non-uubiquitin</u> self-epitope. Mouritsen et al. disclose a fusion protein resulting from the insertion of a single copy of a T cell epitope internally into a single ubiquitin amino acid sequence to produce a fusion protein which is antigenic for the ubiquitin

portion. Mouritsen et al. does not disclose use of a ubiquitin fusion protein to generate an immune response to a non-ubiquitin self-antigen.

Rejection Under 35 USC 103(a)

Claims 82-100 have been rejected under 35 USC 103(a) as unpatentable over van der Zee et al. in view of Vannier et al. More specifically the Patent Office states:

> it would have been obvious to one of ordinary skill in the art at the time of the instant invention to modify ubiquitin fusion proteins disclosed by Vannier et al. to use GnRH as the self epitope as disclosed by van der Zee et al. since GnRH is considered the pivotal regulatory peptide in mammalian reproduction and there is a demand for an effective, low cost means of controlling fertility in domestic animals.

This rejection is respectfully traversed. In the present invention, ubiquitin is used as a scaffold to stabilize and display recombinant self-epitopes in the generation of an immune response to those self-epitopes. Applicants have discovered that ubiquitin fused to an epitope or epitopes, in a defined manner, is useful for the stimulation of an immune response to the fused epitope when administered to an animal. This immune response is highly specific for the fused epitope(s) (even if the epitope is otherwise non-immunogenic), with little to no immune response being generated to the ubiquitin portion of the fusion protein. Importantly, Applicants have further discovered that this process can also be used to generate an immune response to (non-ubiquitin) self-antigens; ubiquitin fused to a heterologous self-epitope in a defined manner is useful for generation of an immune response to that self-epitope. Prior to the present invention, ubiquitin was not recognized in the art as an immunological carrier (meaning that it confers immunogencity to an attached polypeptide) for an otherwise non-immunogenic epitope. Rather, ubiquitin fusion proteins were used in the art to either study the ubiquitin degradation pathway of proteins, or to stabilize

and promote proper folding of a fused protein during synthesis/purification in a bacterial system.

The ability of ubiquitin to function well as an immunological carrier for heterologous self-epitopes could not have been predicted by one of skill in the art with any degree of certainty, from the combined disclosures of Vannier et al. and van der Zee et al. because neither discloses or suggests that ubiquitin confers immunogencity to an otherwise non-immunogenic fused epitope. Vannier et al. teaches ubiquitin fused at it's C-terminus to the extracellular domain (a.a. 23-358) of human FSRH (the fusion being cleavable by ubiquitin specific proteases), and the use of this fusion to generate an immune response to the human FSRH in mice and monkeys. van der Zee et al. teaches the highly immunogenic carrier P-fimbriae fused to the short, non-immunogenic (see first paragraph of van der Zee et al.) GnRH decapeptide, and use of this fusion to produce an immune response to the GnRH decapeptide in an animal. Applicants' discovery of the ability of ubiquitin to confer immunogenicity to an otherwise non-immunogenic epitope was unexpected since ubiquitin is such a highly conserved protein and thus is minimally antigenic itself. It is generally understood in the art is that an immunological carrier functions as such because it is itself highly immunogenic. Given this rule of thumb, prior to the present invention, one of ordinary skill in the art would not have predicted that a ubiquitin fusion protein consisting of ubiquitin and the non-immunogenic GnRH decapeptide (the GnRH decapeptide is specifically described as non-immunogenic in the first paragraph of van der Zee et al.) would have the ability to stimulate an immune response to the heterologous epitope contained therein, because of the minimal antigenicity of ubiquitin. Along those same lines, one of skill in the art would not reasonably conclude that ubiquitin functions in the fusion protein disclosed in Vannier et al. as an immunological carrier for the fused FSHR because the fused FSHR is relatively large (335 amino acids), foreign, and thus likely inherently immunogenic. Prior to the

present invention, one of skill in the art would reasonably conclude that the generation of an immune response to the injected ubiquitin-FSHR fusion protein, taught by Vannier et al. was due to the inherent immunogenicity of the FSHR polypeptide sequences. This conclusion would be further supported by the fact that the Ub-FSHR protein is susceptible to cleavage by ubiquitin specific proteases *in vivo*, and thus the FSHR polypeptide sequences are rapidly cleaved from the ubiquitin sequences upon injection into the animal by ubiquitin-specific proteases present in mammalian tissue. Most importantly, one of skill in the art would not have predicted from the combined disclosures of Vannier et al. and van der Zee et al. that ubiquitin has the ability to function as an immunogenic carrier for a self-epitope since the disclosure of Vannier et al. does not indicate that an immune response was generated to a self-epitope. This is because the FSHR in the ubiquitin-FSHR fusion protein was of human origin, rather than mouse or monkey origin, and thus contained an abundance of non-self epitopes (regions of amino acid sequence not conserved between humans and mice, or humans and monkeys).

Claims 82-100 have been rejected under 35 USC 103(a) as unpatentable over Mouritsen et al. in view of van der Zee et al. More specifically the Patent Office states:

> It would have been obvious to one of ordinary skill in the art at the time of the instant invention to modify ubiquitin fusion proteins disclosed by Mouritsen et al. to use GnRH as the self epitope as disclosed by van der Zee et al. since GnRH is considered the pivotal regulatory peptide in mammalian reproduction and there is a demand for an effective, low cost means of controlling fertility in domestic animals.

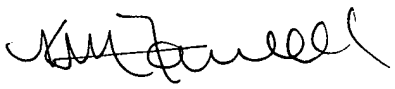
This rejection is respectfully traversed. Applicants' invention differs profoundly from the combined teachings of Mouritsen et al. and van der Zee et al. Mouritsen et al. teach use of a fusion protein resulting from the insertion of a single copy of a T cell epitope internally into a single ubiquitin amino

acid sequence to produce an immune response to ubiquitin. van der Zee et al. teaches use of the highly immunogenic carrier P-fimbriae fused to the short, non-immunogenic (see first paragraph of van der Zee et al.) GnRH decapeptide to generate an immune response to the GnRH decapeptide in an animal. One of skill in the art would not predict with any degree of certainty the generation of an immune response to a self-epitope from immunization with ubiquitin fused to a self-epitope from the combined disclosures of van der Zee et al. and Mouritsen et al. because neither disclosure teaches or suggests that ubiquitin has the ability to function as an immunological carrier for a heterologous epitope, especially a heterologous self-epitope. As discussed above in connection with the rejection of Claims 82-100 as obvious over van der Zee et al. in light of Vannier et al., this ability was not previously appreciated in the art, and the discovery of said ability by Applicants was an unexpected result.

Summary

In light of the above amendment and remarks, reconsideration of the subject patent application is respectfully requested.

Respectfully submitted,



Kevin M. Farrell
Attorney for Applicants
Registration No. 35,505
(207) 363-0558

York Harbor, ME
Dated: 4/16/01

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